evidently increased, suggesting the acidification between the membrane-surface space. Additionally, the degree of the acidification was proportional to the concentration of uncaged ATP ranging from 0.2 μM to 0.7 μM. These results suggest that we successfully detected the proton pumping activity sensitively and quantitatively. As many kinds of Caged compounds and ion sensitive fluorescent dyes are commercially available, this technique can be applied to other ion pumps and transporters.

Mitochondrial & Chloroplast Transport

1539-Pos Board B431

Ran GTPase-Independent and Stereochemical Control of Kinesin-1 and Mitochondrial Motility by Domains of Ran-Binding Protein-2

Paulo A. Ferreira, Hemangi Patil, Kyoung-in Cho.
Duke University Medical Center, Durham, NC, USA.

The microtubule-based motor proteins, dynein and kinesin-1, mediate fast mitochondrial trafficking, but the mechanisms underlying the regulation of mitochondrial motility are ill-defined. The Ran-binding protein 2 (RanBP2) is a pleiotropic and multimodular protein, which couples directly with the kinesin-1 isoforms, KIF5B/KIF5C, via its tripartite domains, the kinesin-binding domain (KBD) and the Ran GTPase-binding domains, RBD_2 and RBD_3. The coupling of RBD_2-KBD-RBD_3 to kinesin-1 activates its ATPase activity ~30-fold and with activation kinetics that is biphasic and cooperative. Here, we employ structure-function, biochemical, kinetic and cell-based assays with time-lapse live-cell microscopy of over 260,000 mitochondrial motility-related events to probe the interplay between Ran GTPase and RBD_2-KBD-RBD_3 on kinesin-1 activation and mitochondrial motility. We uncover mutually exclusive subdomains in RBDs toward Ran GTPase binding, kinesin-1 activation and modulation of mitochondria motility. The RBDs exhibit Ran-GTP-independent, subdomain and stereochemical-dependent discrimination on the biphasic activation kinetics of kinesin-1 or regulation of mitochondrial motility. Remarkably, RBD_2-KBD-RBD_3 and KBD alone exert opposing effects on the equilibrium between the stationary and motile phases of mitochondria and multiple biophysical parameters of mitochondrial motility. Further, the regulation of the bidirectional transport of mitochondria by either RBD_2-KBD-RBD_3 or KBD is highly coordinated, since their effects are accompanied always by changes in motile biophysical parameters of opposite-polarity. These studies uncover Ran GTPase-independent antagonizing and multimodal mechanisms of kinesin-1 activation and regulation of mitochondrial motility by distinct domains of RanBP2. Further, they open new venues toward the pharmacological harnessing of mechanisms regulating kinesins, mitochondrial motility or RanBP2 in a variety of disparate disorders.

1540-Pos Board B432

MICU1 is an Essential Gatekeeper for MCU-Mediated Mitochondrial Ca^{2+} Uptake that Regulates Cell Survival

Muniswamy Madesh[1], Karthik Mallilankaraman[1], Patrick Doonan[1], Cesar Cardenas[2], Harish C. Chandramoorthy[1], Marioly Muller[2], Russell Miller[2], Nicholas Hoffman[1], Rajesh Gandhirajan[1], Morris Birnbaum[2], Brad Rothber[1], Don-On Daniel Mak[2], James Kevin Foskett[2].
[1]Temple University School of Medicine, Philadelphia, PA, USA, [2]University of Pennsylvania, Philadelphia, PA, USA.

Mitochondrial Ca^{2+} uptake is mediated by an inner membrane Ca^{2+} channel called the uniporter. Ca^{2+}uptake is driven by the considerable voltage present across the inner membrane ($\Delta\Psi_m$) generated by proton pumping by the respiratory chain. Mitochondrial matrix Ca^{2+} concentration ($[Ca^{2+}]_m$) is maintained 5-6 orders of magnitude lower than its equilibrium level, but the molecular mechanisms for how this is achieved are not clear. Here we demonstrate that the mitochondrial protein MICU1 is required to preserve normal $[Ca^{2+}]_m$ under basal conditions. In its absence, mitochondria become constitutively loaded with Ca^{2+}, triggering excessive reactive oxygen species generation and sensitivity to apoptotic stress. MICU1 interacts with the uniporter pore-forming subunit MCU and sets a Ca^{2+} threshold for mitochondrial Ca^{2+} uptake without affecting the kinetic properties of MCU-mediated Ca^{2+} uptake. Thus, MICU1 is a gatekeeper of MCU-mediated mitochondrial Ca^{2+} uptake that is essential to prevent mitochondrial Ca^{2+} overload and associated stress.

1541-Pos Board B433

Translocation of Knotted Proteins into Mitochondria

Piotr Szymczak.
University of Warsaw, Warsaw, Poland.

In recent years a surge of interest has arisen in properties and function of knotted proteins. As more and more knotted structures are discovered in the Protein Data Bank, it becomes increasingly important to understand how, if at all, the non-trivial topology affects the protein's function in the cell. In particular, it has been hypothesized that the presence of a knot in the polypeptide backbone may affect the ability of knotted proteins to be degraded in proteosome or translocated through the intercellular membranes, e.g. during import into mitochondria. In these processes, the translocating proteins typically have to pass through constrictions that are too narrow to accommodate folded structures, thus translocation must be coupled to protein unfolding. However, as shown in a number of theoretical and experimental studies the protein knots get tightened under the tension. The radius of gyration of the tight knot is about 7-8 Angstrom, whereas the diameters of the narrowest constriction of the mitochondrial pores are in the 12-15 Angstrom range, making it possible for the knots to get stuck during the translocation process. In this communication, we report the result of molecular dynamics simulations of knotted protein translocation which show how such topological traps might be prevented by using a pulling protocol of a repetitive, on-off character. Such a repetitive pulling is biologically relevant, since the mitochondrial import motor, like other ATPases transform chemical energy into directed motions via nucleotide-hydrolysis-mediated conformational changes, which are cyclic in character. This research has been supported by the Polish NCN grant N N202 055440.

1542-Pos Board B434

On the Role of Positively Charged Residues of TM2 Domain in the Chloride Transport of Human UCP2

Tuan Hoang[1], Tijana Matovic[2], James Parker[2], Matthew D. Smith[2], Masoud Jelokhani-Niaraki[2].
[1]University of Guelph, Guelph, ON, Canada, [2]Wilfrid Laurier University, Waterloo, ON, Canada.

Located in the inner mitochondrial membrane, uncoupling proteins (UCPs) dissipate the proton electrochemical gradient causing reduction in the rate of ATP synthesis. Among five human UCP homologues, UCP2 is unique with its ubiquitous expression in various tissues. This important feature has been attributed to UCP2's multiple physiological roles in tissues, including its involvement in protective mechanisms against oxidative stress, glucose and lipid metabolisms. Despite numerous physiological studies, UCP2 function in cell remains poorly understood. UCP2 proton transport is regulated by purine nucleotides such as ATP, ADP, GTP and GDP. In addition, UCP2 has also been observed to transport chlorides and other small anions. Identification of the key amino acid residues in UCP2 in proton, anion transport and regulation will help determine the protein's mechanism of action in cells. It has been established that positively charged residues on transmembrane helix 2 (TM2) of UCP1 and UCP2 are crucial for chloride transport. However, a full understanding of the transport mechanism is yet to be achieved. More importantly, some of these residues are also involved in the UCP2 proton transport regulation. To further understand the ion transport of UCP2, four TM2 mutants have been made (R76Q, R88Q, R96Q, and K104Q). Over-expressed proteins were purified and reconstituted into liposomes for structural and functional studies. All mutants share an overall helical conformation with wtUCP2. using anion-sensitive fluorescent probes, proton and chloride transport of UCP2 mutants are examined to determine the effect of each mutation on the ion transport of UCP2. In addition, Mant-modified purine nucleotide will be used to study the binding of UCP2 and its mutants to purine nucleotides. Overall, the outcome of this study will provide a more detailed molecular image of UCP2 ion transport mechanism.

1543-Pos Board B435

Regulation of Mitochondrial Ca^{2+} Dynamics by Inorganic Phosphate

An-Chi Wei, Ting Liu, Raimond L. Winslow, Brian O'Rourke.
Johns Hopkins University, Baltimore, MD, USA.

The mitochondrial Ca^{2+} uniporter (mCU) is the main influx pathway for the uptake of mitochondria Ca^{2+} and plays an important role in regulating energy production as well as cell life and death. In our previous study, we reported two modes of the mCU (mode1 and mode 2) that have different properties with respect to Ca^{2+} affinity (mode1 > mode 2) and Ru360 sensitivity (mode 1 < mode 2). This study further investigates the role of concomitant inorganic phosphate (Pi) transport on mitochondrial uptake of Ca^{2+} mediated by the mCU in isolated cardiac mitochondria loaded with mitochondrial and extra-mitochondrial Ca^{2+} fluorescent dyes (Fura-FF and Calcium Green-5N). In Pi-depleted mitochondria, the maximal Ca^{2+} uptake rate is shown here to be limited by extramitochondrial Pi. The mitochondrial Ca^{2+} uptake rate was accelerated in a concentration-dependent manner by phosphate concentrations ranging from 0.01 mM to a maximum at ~0.1 mM. The effect of Pi on mode 2

Ca^{2+} uptake was largely inhibited in the presence of the mitochondrial phosphate carrier inhibitor N-ethylmaleimide; however, mode 1 uptake was still observed, i.e., bulk Ca^{2+} uptake through mCU mode 2 was more Pi- dependent than mode 1. These experiments demonstrate another distinction between mCU modes 1 and 2 and contribute to an understanding of their possible physiological roles in mitochondrial function as either a signal for regulating energetics or as a Ca^{2+} sink.

Oxidative Phosphorylation & Mitochondrial Metabolism

1544-Pos Board B436

Role of Mitochondrial Morphology in Bioenergetics

Hakjoo Lee[1], Bong Sook Jhun[2], **Yisang Yoon**[1].
[1]Georgia Health Sciences University, Augusta, GA, USA, [2]Thomas Jefferson University, Philadelphia, PA, USA.

Mitochondria in cells undergo constant morphological changes mainly through fission and fusion. However, functional significance of mitochondrial fission and fusion is not fully understood. To test the importance of mitochondrial morphology in maintaining mitochondrial function, first, we used glucose-stimulated insulin secretion in pancreatic β-cells as an experimental model because insulin secretion upon elevated plasma glucose concentration requires intact mitochondrial function. Increased ATP production in mitochondria from glucose metabolism induces plasma membrane depolarization and subsequent increase of cytosolic Ca^{2+} triggers insulin exocytosis. We found that glucose stimulation of the β-cell line INS-1E induces transient mitochondrial shortening and recovery. Inhibiting mitochondrial fission by expressing the dominant-negative fission mutant DLP1-K38A abolished the dynamic change of mitochondrial morphology in glucose stimulation. Importantly, we discovered that abolition of the glucose-induced mitochondrial morphology change suppresses glucose-stimulated insulin secretion. Measuring respiration under fission inhibition showed an increase of mitochondrial uncoupling, and thus significantly diminished the mitochondrial ATP production in response to glucose stimulation. Further evaluation of mitochondrial membrane potential in primary hepatocytes revealed that inhibition of mitochondrial fission induces large-scale fluctuations of the potentiometric fluorescence in mitochondria within cells. Frequencies and intervals of the fluorescence oscillation were random and insensitive to inhibitors of anion channels and mitochondrial permeability, and superoxide scavenger. This suggests that the fission inhibition-induced fluctuation of the inner membrane potential is a previously unrecognized unique phenomenon. These observations demonstrate that inhibition of mitochondrial fission induces a large-scale fluctuation of the mitochondrial inner membrane potential, which is functionally reflected in mitochondrial uncoupling. Taken together, our findings indicate that mitochondrial fission plays a role in regulating the coupling efficiency of oxidative phosphorylation.

1545-Pos Board B437

Effects of Reactive Oxygen Species on NFAT Activation and Translocation in Adult Rabbit Ventricular Myocytes

Stefanie Walther, Joshua N. Edwards, Lothar A. Blatter.
Rush University Medical Center, Chicago, IL, USA.

Nuclear factor of activated T cells (NFAT) transcription factors play a key role during cellular remodeling associated with cardiac hypertrophy and heart failure (HF). Evidence suggests that reactive oxygen species (ROS) are integral to the progression of cardiac hypertrophy and HF. Therefore, we aimed to determine the role of ROS in the activation and translocation of NFAT.

Adult rabbit ventricular myocytes were infected with recombinant adenoviruses encoding for NFAT-GFP fusion proteins (isoforms NFATc1 and NFATc3). The subcellular distribution of NFAT was quantified as the ratio of NFATnuc to NFATcyt fluorescence (RNFAT) and nuclear-cytosolic NFAT translocation was expressed as changes of RNFAT. Under basal unstimulated conditions, NFATc3 was predominantly localized in the cytoplasm, whereas NFATc1 displayed a nuclear localization.

Acute exposure to the inhibitors of oxidative phosphorylation Rotenone, Antimycin A, Oligomycin and FCCP resulted in the activation and translocation of NFATc1 (60 - 145 % increase of RNFAT) into the nucleus. These inhibitors did not induce translocation of NFATc3; however, exposure to Hydrogen Peroxide (H2O2) or Ruthenium Red (inhibitor for the mitochondrial Ca uniporter), resulted in the activation and translocation of NFATc3 (but not NFATc1). The H2O2-induced NFATc3 translocation was attenuated in the presence of the antioxidant N-acetylcysteine.

These data identify a ROS-induced activation and translocation of NFAT in adult ventricular myocytes.

1546-Pos Board B438

Unpolymerized βII Tubulin in Regulation of Mitocondrial Function in Muscle Cells

Minna Karu[1,2], Rafaela Bagun[3], Madis Metsis[1], Alexei Grichine[3], Kersti Tepp[2], Tuuli Käämbre[2], Valdur Saks[2,3], Rita Guzun[3].
[1]Tallinn University of Technology, Tallinn, Estonia, [2]National Institute of Chemical Physics and Biophysics, Tallinn, Estonia, [3]Joseph Fourier University, Grenoble, France.

The importance of microtubular system in shaping the organization of intracellular energy metabolism and regulating mitochondrial functioning is becoming increasingly more evident. Our previous studies with cardiac cells have shown that regulation of mitochondrial outer membrane (MOM) permeability for ADP by dimeric αβ-tubulin is important for efficient cross-talk between mitochondria and contraction apparatus through phoshocreatine energy transfer pathway. This regulation was specifically related with tubulin isoform βII after showing its mitochondrial localization in cardiac cells and verifying its concomitant expression with mitochondrial creatine kinase (MtCK). However the exact mechanism of this regulation is still rather elusive and studied mainly in cardiac cells. To determine if βII tubulin expression is specific only to oxidative muscle cells with high MtCK activity and to gain further insight to the role of βII tubulin in energy metabolism, we have analyzed the relationship between βII tubulin expression, mitochondrial respiration regulation and their intracellular positioning in striated muscles with different metabolic phenotype. In this study we provide further proof for the functional importance of βII tubulin in regulation of mitochondrial respiration in striated muscles. We show that both oxidative and glycolytic muscles express βII tubulin, but the presence of unpolymerized βII tubulin is significantly lower in glycolytic muscle cells concomitant with higher MOM permeability for ADP. Analysis of mitochondria and βII tubulin localization reveals that in oxidative muscle cells mitochondria are positioned in close vicinity to βII tubulin with high degree of colocalization which is much less prevalent in glycolytic muscles. Together our results show that βII tubulin displays both structural and regulatory role in striated muscle cells and its distribution and polymerization level has direct impact on regulation of mitochondrial ADP sensitivity and efficiency of mitochondria coupling with contraction apparatus.

1547-Pos Board B439

Substrate Oxidation Control of Respiratory Rates in Primary Hepatocytes

Anil Noronha Antony, Cynthia Moffat, Aditi Swarup, Erin Seifert, Jan B. Hoek.
Thomas Jefferson Universiy, Philadelphia, PA, USA.

The goal of our studies is a bioenergetics profiling of primary rat hepatocytes using the Seahorse-XF analyzer in order to assess adaptation in response to metabolic stress or disease. Cellular oxygen consumption rates (JO2) were compared in enriched medium (DMEM) vs a balanced salt solution (HBSS) without added substrates. Hepatocytes exhibited higher basal JO2 in DMEM compared to HBSS and showed a proportional increase in oligomycin-insensitive JO2. The fractional increase in JO2 by uncoupler was higher in DMEM than in HBSS, presumably due to substrate supply by amino acids present in DMEM. These data suggests that substrate oxidative pathways exert significant control over basal and uncoupled respiration rates in primary rat hepatocytes. To further test this hypothesis, we assessed JO2 under different substrate conditions, in DMEM or HBSS medium. Addition of mono-methylsuccinate (MMS), a mitochondrial Complex III substrate, resulted in a large concentration- dependent stimulation of basal JO2 of hepatocytes in HBSS but a more limited stimulation in DMEM, likely reflecting availability of alternate substrates. In DMEM, physiological glucose concentrations (11mM) had little stimulatory effect, while higher concentrations (25mM) inhibited O2 uptake, thus exhibiting a ''Crabtree-like'' effect, which was not overcome by uncoupler treatment. This inhibitory effect of high glucose was not evident in HBSS, where basal JO2 increased with higher concentrations of glucose. Oligomycin-insensitive JO2, as a fraction of basal O2 uptake remained similar under all substrate conditions in DMEM and HBSS, apart from a small decrease at the highest MMS concentration. These results suggest a significant control exerted by substrate oxidative pathways over basal and uncoupler-stimulated respiration rates in primary rat hepatocytes. Electron supply may limit the rate of uncoupled respiration in hepatocytes, underestimating the reserve capacity in the electron transport chain. Supported by NIH grants AA018873 and AA017261.